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PHYSIOLOGICAL PROPERTIES OF DISSOCIATED MUSCLE FIBRES OBTAINED FROM INNERVATED AND DENERVATED ADULT RAT MUSCLE

BY ANNE BEKOFF* AND W. J. BETZ

From the Dept. of Physiology, University of Colorado School of Medicine, Denver, Colorado 80220, U.S.A.

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SUMMARY

1. Adult rat flexor digitorum brevis muscles were dissociated by treatment with collagenase and trituration. Several hundred isolated fibres were obtained from each muscle.

2. Most isolated fibres appeared to be intact as judged by some morphological and physiological criteria, although resting membrane potentials were about -60 mV, which is somewhat lower than normal.

3. A small percentage of the muscle fibres were branched.

4. Acetylcholine sensitivity was measured iontophoretically. The sensitivity fell abruptly outside the margin of the end-plate. Extrajunctional sensitivity was detected on all fibres, and declined smoothly away from the end-plate to an undetectable level over a distance of about 200 μm. On a few fibres, ACh sensitivity was mapped circumferentially from the end-plate. It appeared to decline with distance in a manner similar to the longitudinal sensitivity gradient.

5. Fibres dissociated from muscles denervated a week earlier were sensitive to ACh everywhere on their surfaces.

INTRODUCTION

The spatial distribution of acetylcholine (ACh) receptors on skeletal muscle fibres has been determined with increasingly fine resolution in recent years. Two advances in iontophoretic techniques are especially noteworthy: first, use of high resistance ACh micropipettes reduces leakage of ACh and consequent desensitization of receptors (Harris, Kuffler & Dennis, 1971; Dreyer & Peper, 1974*a*) and secondly, preparations (notably certain frog and snake muscles) have been discovered which permit high quality optical resolution, so that subcellular structures

* Present address: Department of E.P.O. Biology, University of Colorado, Boulder, Colorado, U.S.A.

can be observed and microelectrodes can be positioned with high accuracy under direct visual control (McMahan, Spitzer & Peper, 1972; Peper & McMahan, 1972). For instance, with such techniques, Kuffler & Yoshikami (1975) showed that ACh sensitivity drops precipitously (by fiftyfold or more) within a 2–3 μm distance beyond the edge of end-plates in frog and snake muscles. In addition, Dreyer & Peper (1974*c*) have shown that, a few days after denervation, frog muscle extrajunctional receptors are not uniformly distributed. That is, distinct and separate patches of sensitive membrane appear and also, a perijunctional minimum of sensitivity can sometimes be observed.

In mammalian muscle, the existence of extrajunctional receptors on innervated fibres (Miledi, 1960) and the appearance of new receptors after denervation (Axelsson & Thesleff, 1959) have been well documented. However, the relatively poor visual resolution afforded by mammalian muscle preparations has not permitted the kind of detailed iontophoretic mapping which is possible with frog and snake muscles. This paper describes a simple technique for enzymatically dissociating fibres in adult rat toe muscle. The excellent visual resolution afforded by the isolated fibres has permitted detailed mapping of ACh sensitivity, with a spatial resolution of several microns. Experiments were performed on fibres obtained from both innervated and denervated muscles.

METHODS

Flexor digitorum brevis is located on the plantar aspect of the hind foot, and consists of a few thousand short muscle fibres which are divided into three slender muscles whose actions are to flex the second, third and fourth digits. Muscles were dissected from freshly killed adult female Sprague-Dawley rats (100–150 g) and incubated approximately 2·5 hr in Ca-free minimal Eagle's Medium (M.E.M., Difco) containing 0·3 % collagenase (Sigma, Type I) in a 37° C incubator with a 5 % CO_2–95 % O_2, water-saturated atmosphere. The muscles were then rinsed in M.E.M. with 10 % horse serum (Colorado Serum Co.), 2 % chick embryo extract, glutamine, 0·29 g/l., streptomycin, 0·52 g/l., penicillin, 17 mg/l., $NaHCO_3$, 2 g/l., and $CaCl_2$, 0·2 g/l. Next the softened tendons were gently pulled apart and the smaller clusters of fibres were triturated gently with a Pasteur pipette.

Isolated fibres were transferred to a glass-bottomed chamber and the bathing medium was covered with mineral oil. The chamber was placed on the stage of a Reichert inverted microscope fitted with interference-contrast optics. Experiments were performed at room temperature.

Electrophysiology. Intracellular electrodes were filled with 4 M-K acetate and had resistances of 80–200 MΩ. Input resistance was measured with two closely spaced intracellular electrodes, one for injecting square pulses of current, the other for measuring the resulting change in membrane potential. Iontophoretic electrodes were filled with 3 M ACh chloride and had resistances of 150–400 MΩ. Braking currents of 1–4 nA were required to prevent measurable depolarization when the tip of the ACh electrode was placed at an end-plate. Iontophoretic current was monitored from the output of an operational amplifier which clamped the bath at

virtual ground. Recording and iontophoretic electrodes were never separated by more than 150 μm during an experiment. Sensitivities were calculated as mV peak depolarization divided by iontophoretic charge (nC). Identical ACh pulses were used during individual mapping runs, with the dose adjusted at the outset to produce a peak depolarization of 2–6 mV. ACh pulse durations in different runs ranged from 2 to 10 msec; total coulombs delivered ranged up to 200 pC. The minimum detectable sensitivity was about 0·5 mV/nC.

Denervation. Rats were anaesthetized with ether. An incision was made on the medial surface of the ankle, and the medial and lateral plantar nerves were exposed. Both were cut. The incision was closed with a skin clip. The denervated flexor digitorum brevis muscles were dissociated 1–13 days later.

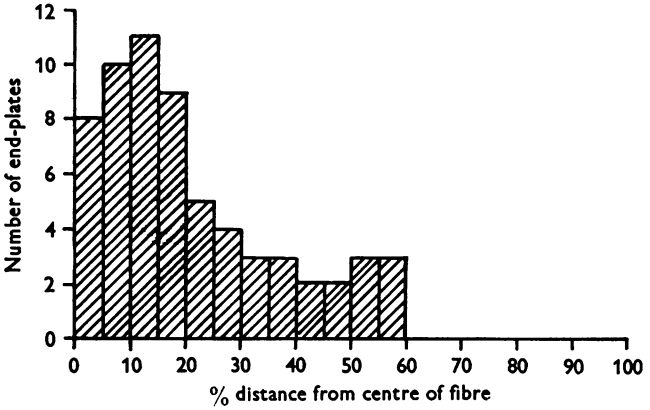
RESULTS

Morphological observations. The dissociation procedure yielded hundreds of individual fibres, most of which appeared to be intact (Pl. 1). Under high power (780 ×) Nomarski optics, cross striations were clearly visible, as were nuclei, which usually bulged slightly from the surface and appeared to be distributed uniformly along the length of the fibres. Which, if any of the nuclei belonged to satellite cells was not determined. End-plates were easily identifiable as circular patches about 20–25 μm in diameter, although two or three closely spaced nuclei, viewed on edge, could be mistaken for an end-plate. Positive identification of end-plate location was easily obtained by observing a twitch in response to iontophoretically applied ACh. Structures within the margin of the end-plate were quite variable in appearance. Some end-plates, for instance, appeared as flat patches (Pl. 1*C*) while others bulged from the surface (Pl. 1*D*). Possibly, the former were bare end-plates from which nerve terminal and Schwann cell had been dissociated, while the latter were covered with cellular debris, adherent fragments of nerve and Schwann cells. The ends of the fibres were thrown into folds, characteristic of the tendinous insertions of skeletal muscle (Pl. 1*E*).

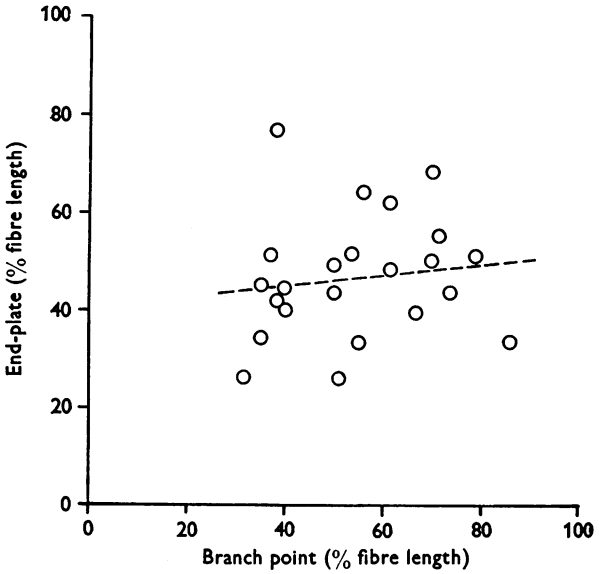
The fibres varied in length, most falling between 700 and 1200 μm (mean $\pm$ S.D. = 880 $\pm$ 106 μm). Diameters ranged from 20 to 50 μm, most falling between 30 and 40 μm. End-plates tended to be near the middle of the fibres (Text-fig. 1), few occurring outside the central half of the fibres. No fibres with more than one plate were observed.

Branched fibres. A small number of fibres, accounting for about 1 % of all fibres, were branched (Pls. 2 and 3). Twenty-six of these were examined in some detail. Each had a single end-plate (identified visually and confirmed by an ACh-evoked twitch). About two thirds of the end-plates were on the main trunk, the remainder on one of the branches. For any given branched fibre, the end-plate and branch point locations were apparently unrelated, as shown in Text-fig. 2.

In any fibre, the two branches were of approximately the same diameter and length (one exceptional fibre was observed, in which branch diameters were 15 and 36 μm). Branch diameters were smaller by about one third



Text-fig. 1. Histogram showing the location of end-plates along the length of sixty-three isolated muscle fibres. Zero on the abscissa represents the middle of the fibre, 100 represents the end of the fibre nearer the end-plate.



Text-fig. 2. The location of end-plates (ordinate) is plotted against the location of branch points (abscissa) for twenty-three singly-branched muscle fibres. The dashed line was obtained by linear regression, and the correlation coefficient is 0·13, which suggests that end-plate locations are not related to branch point locations.

than the diameter of the main trunk. This suggests that the total cross-sectional area is approximately constant along the length of the fibre.

In addition to the singly branched fibres, three doubly branched fibres were observed. An example is shown in Pl. 3*C*. Finally, one fibre, in addition to being branched (singly), had what appeared to be a cleft splitting it longitudinally for a distance of about 100 μm (Pl. 3*D*). The cause of these different forms of branching is not known. They may reflect incomplete fusion of embryonic precursors of muscle fibres (myotubes). Alternatively, they may be due to splitting of normal fibres, which has been shown to occur in muscle overloaded by tenectomy of its synergists (Hall–Craggs, 1970) and also following denervation (Miledi & Slater, 1969).

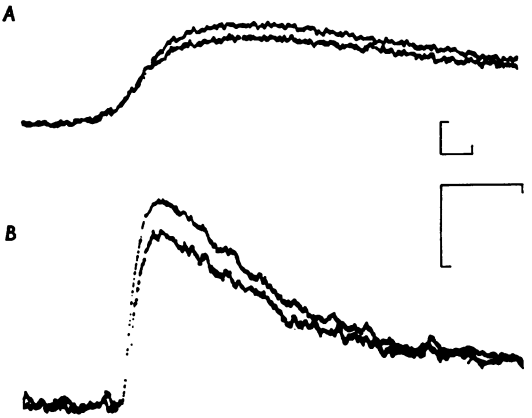
Electrophysiology. Resting membrane potentials in general were lower than the 70–80 mV range observed in undissociated mammalian muscles (Albuquerque & Thesleff, 1968; Albuquerque & McIsaac, 1970). Fibres which appeared to be intact by visual criteria had resting potentials ranging from -40 to -70 mV. Those with resting potentials less than -50 mV were not studied further. The mean ($\pm$ S.D.) of all fibres studied in detail was $-59{\cdot}0$ ($\pm 5{\cdot}0$) mV. The short length of these fibres suggested that membrane electrical parameters could be treated by a short cable model with open circuited ends. Input resistance (R_{in}) was measured at the middle of a number of fibres; values averaged $1{\cdot}0 \pm 0{\cdot}4$ (S.D.) MΩ. For a short cable,

$$R_{in} = \frac{4R_i\lambda}{\pi d^2} \coth (L/2\lambda), \tag{1}$$

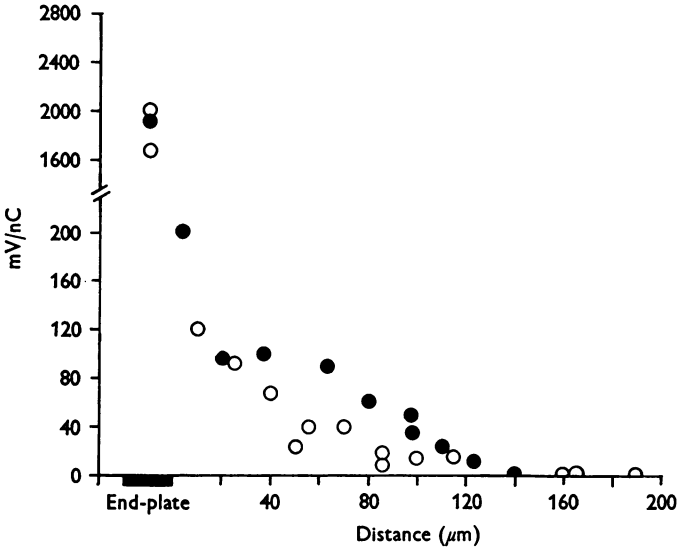
where R_i = specific internal resistance, d = diameter, L = fibre length, and $\lambda = 1/2 \sqrt{(d.R_m)/R_i}$, where R_m = specific membrane resistance (see Jack, Noble & Tsien, 1975). Assuming $R_i = 100\,\Omega$ cm, and using the observed average values of d (40 μm), L (880 μm) and input resistance (1·0 MΩ), R_m is calculated to be 500 Ω cm^2. This value is in good agreement with that obtained from long fibres in undissociated mammalian muscle (Albuquerque & Thesleff, 1968; Albuquerque & McIsaac, 1970). Using this value of R_m, the expected decrement of steady-state potential change produced by current injection with distance can be calculated from

$$\frac{V_x}{V_0} = \frac{\cosh (L/2\lambda - x/\lambda)}{\cosh (L/2\lambda)}, \tag{2}$$

where x = distance between middle of fibre (the site of current injection) and recording electrode (Jack *et al.* 1975). Using average values of L and λ, the potential change recorded at the tendon should be about 83 % of that at the middle. Experimental results agreed with this prediction, as



Text-fig. 3. ACh potentials recorded simultaneously with two recording electrodes, one near the end-plate (larger responses) and the other near the end of a muscle fibre (smaller responses). ACh was applied at the end-plate. *A*, slow responses with the iontophoretic pipette several microns from the end-plate. *B*, faster responses with the iontophoretic pipette closer to the end-plate. Scales represent 2·5 mV and 25 msec.



Text-fig. 4. ACh sensitivity (ordinate) is plotted against distance from the end-plate. Open and filled circles represent data from two different fibres dissociated from control (innervated) muscles.

shown in Text fig. 3. ACh was applied at the end-plate, and depolarizations were recorded simultaneously with two intracellular micro-electrodes, one at the end-plate and the other 380 μm away, near the end of the

fibre. The decrement in peak potential change was 10–15 % over the 380 μm distance (Text-fig. 3*A*). When the ACh electrode was moved closer to the end-plate, a faster rising potential was produced (Text-fig. 3*B*), and the decrement was slightly greater (usually 15–20 %). The greater decrement with the faster potential probably reflects incomplete charging of membrane capacitance along the length of the fibre. In summary, while these fibres were not isopotential, spatial decrement was quite small. In the experiments described below, any decrement was negligible since recording and ACh electrodes were never separated by more than 150 μm.

ACh sensitivity. End-plates were highly sensitive to iontophoretically applied ACh. Sensitivities ranged from 500 to 5000 mV/nC. Rise times of ACh potentials were as short as 3–4 msec. At some end-plates, responses varied considerably with slight (2–4 μm) lateral movements of the ACh electrode, but it was often difficult to reproduce these results, or to correlate spots of relatively high or low sensitivity with any observable structure. These variations of sensitivity within the end-plate borders probably reflect, at least in part, the variable bits of nerve and Schwann cell debris adhering to end-plates. In general, sensitivity near the edge of an end-plate was similar to that in the centre which suggests that there was no over-all gradient in the end-plate region. However, gradients over smaller distances within the end-plate area cannot be ruled out by these experiments.

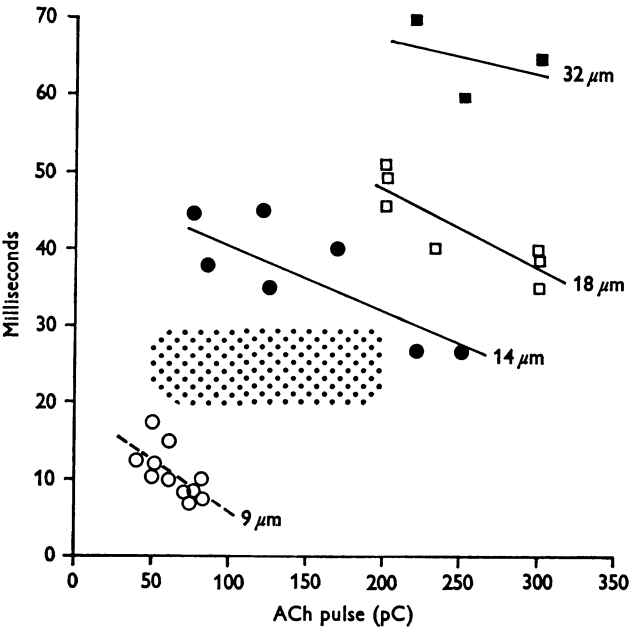
ACh sensitivity was also detected outside the end-plate region. This extra-junctional sensitivity fell progressively from the end-plate region to less than 2 mV/nC at a distance of 150 μm (Text-fig. 4). No sensitivity could be detected at distances greater than about 250 μm, or at the ends of the fibres. These results are similar to observations by Miledi (1960) on normal muscle fibres in rat diaphragm.

It was of interest to measure extrajunctional sensitivity as close to the end-plate as possible. The difficulty of course is that as the end-plate is approached with the iontophoretic pipette, the risk increases that some of the ACh released will diffuse to and activate end-plate receptors. If this occurs to any measurable extent before the peak of the extrajunctional response occurs, then a spuriously large extrajunctional sensitivity will be calculated.

Kuffler & Yoshikami (1975) devised an elegant method for temporally separating junctional and extrajunctional contributions to a response. The experiment, performed on frog and snake muscle preparations, involved comparing responses obtained with and without an anticholinesterase present. The drug potentiated only the contribution of end-plate receptors. Their results showed clearly, for instance, that at a distance of 12 μm, the latency of the end-plate response was about 25 msec with a 41 pC ACh pulse (Fig. 8, Kuffler & Yoskikami, 1975). Analogous experiments

were not possible with the dissociated flexor digitorum brevis muscle fibres, because the collagenase treatment removes the cholinesterase from the end-plate region (Hall & Kelly, 1971; Betz & Sakmann, 1973).

In the present study, a different kind of experiment was performed (cf. Peper & McMahan, 1972). The results indicated that extrajunctional responses were not contaminated by activation of end-plate receptors if the ACh pipette was at least 15–20 μm from the end-plate. The experiment involved measuring the latency of end-plate responses as a function of distance (between the end-plate and ACh pipette) and dose of ACh. As expected, the measured latency decreased with smaller distances or larger



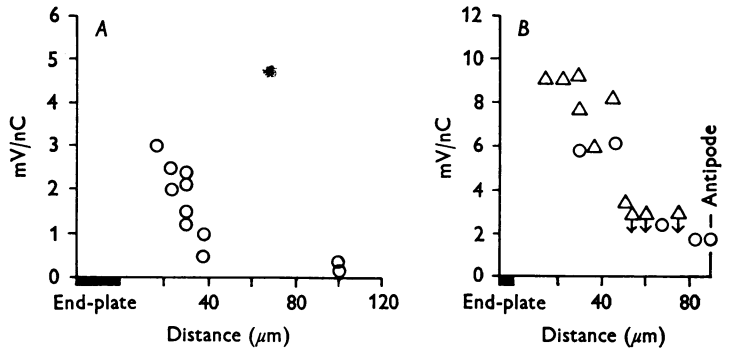
Text-fig. 5. This graph shows the dependence of the latency of ACh potentials (ordinate) on distance (between the ACh pipette tip and the end-plate) and on iontophoretic pulse strength (abscissa). ACh was applied at four positions directly in front of the end-plate (i.e. along a line passing through the end-plate and perpendicular to the fibre axis): open circles, 9 μm in front of end-plate; filled circles, 14 μm; open squares, 18 μm; filled squares, 32 μm. Lines were drawn by linear regression. The latency increases with increasing distance and with decreasing pulse strength. The stippled area represents parameters for measuring extrajunctional sensitivity: ACh potentials had rise times of 20–30 msec (ordinate) and iontophoretic pulses of 50–200 pC (abscissa) were typically used. For distances greater than 14 μm from end-plate, the latency of the end-plate response was longer than the rise time of the extrajunctional response. Therefore, diffusion of ACh to end-plate receptors did not contaminate extrajunctional sensitivity measurements.

ACh pulses (Fig. 5). The experiments were performed by positioning the ACh pipette at different points along a line passing through the end-plate and perpendicular to the fibre axis. At each point, different amounts of ACh were applied. Probably most, if not all, of the observed responses were due to activation of end-plate receptors. The graph in Text-fig. 5 shows the dependence of latency (ordinate) on iontophoretic dose (abscissa) for different distances between the end-plate and ACh pipette. The shaded area in Text-fig. 5 indicates the usual conditions for mapping *extra*junctional sensitivity. The time to peak of extrajunctional responses recorded far (> 50 μm) from the end-plate was 20–30 msec (ordinate) and the usual iontophoretic pulses contained 50–200 pC (abscissa). As can be seen from the graph, if the ACh pipette was about 14 μm or more from the end-plate, the latency of the end-plate response was greater than the time to peak of the extrajunctional response. Therefore, beyond this distance the peak responses provided an accurate measure of extrajunctional sensitivity, uncontaminated by end-plate receptor activation. In summary, it appears that extrajunctional sensitivity could be measured accurately at distances greater than 15–20 μm from the end-plate. A further observation supporting this conclusion was that as the end-plate was approached laterally (i.e. while mapping extrajunctional membrane sensitivity), the rise times and half-decay times of the ACh potentials were relatively constant until the pipette was about 10 μm from the end-plate. At separations smaller than 10 μm, unmistakable signs of end-plate responses were observed as the half-decay time and rise time became prolonged (cf. Dreyer & Peper, 1974*b*; Kuffler & Yoshikami, 1975).

Sensitivities measured 15–20 μm from the end-plate in different fibres ranged from 2 to 120 mV/nC. Part of this variability is probably due to differences in ACh pipette transfer characteristics. At increasing distances from the end-plate, the sensitivity declined smoothly and progressively; no isolated spots of high or low sensitivity were found. At a distance of 150 μm from the end-plate, the sensitivity was usually less than 1–2 mV/nC, and in some fibres the spatial decay was even more abrupt, sensitivity falling to undetectable levels 80–100 μm from the end-plate. No fibres, however, were found without any extrajunctional sensitivity.

The rather steep spatial decrement of extrajunctional sensitivity along the longitudinal fibre axis suggested that it might be possible to detect a similar circumferential gradient from the end-plate, if one existed. Unfortunately, the small diameters of the fibres (30–45 μm), coupled with a 15 μm limit of approach to the end-plate, meant that a decrement in sensitivity would have to be detected over a distance of only 15–30 μm. This was too small a distance to establish reliably a small difference in sensitivity, either circumferentially or longitudinally. However, one large

branched muscle fibre was found which was optimally suited for such an experiment. The end-plate was viewed on edge, and was located at the branch point, where the diameter was about 80 μm. ACh sensitivity was mapped in detail around the end-plate, both longitudinally (Text-fig. 6*A*) and circumferentially (Text-fig. 6*B*). In this fibre, a clear circumferential gradient was observed; the sensitivity fell from about 10 mV/nC (at 20 μm) to about 2 mV/nC (at 80 μm). This was similar to the longitudinal sensitivity gradient.

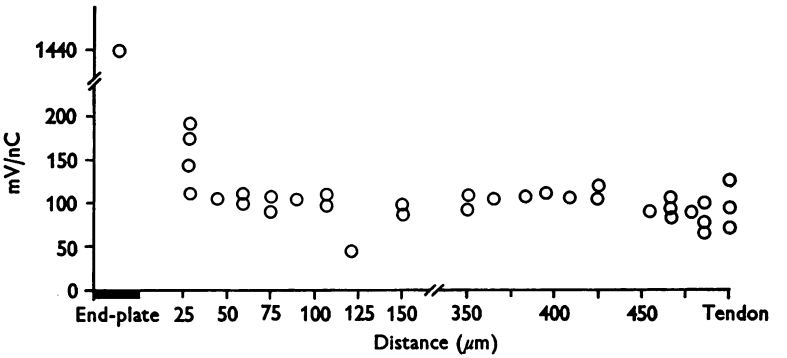


Text-fig. 6. ACh sensitivity (ordinate) mapping longitudinally (*A*) and circumferentially (*B*) from the end-plate. This was a large, branched fibre with the end-plate located at the branch point. Circles: 525 pC ACh pulse; triangles: 120 pC ACh pulse.

Denervated fibres. Experiments like those described above were also performed on dissociated fibres from muscles which had been denervated 1–13 days earlier. Viewed with Nomarski optics, the fibres were indistinguishable from control (not denervated) fibres, except that end-plates were somewhat less distinct and thus more difficult to identify in fibres denervated a week or more. The number and distribution of nuclei appeared unchanged, in agreement with Cardasis & Cooper (1975).

The most extensive physiological measurements were made on fibres denervated seven days earlier. Resting potentials were $-62 \pm 5{\cdot}9$ mV (mean $\pm$ S.D.), which was similar to control values ($-59 \pm 5{\cdot}0$ mV) and to values obtained from undissociated, denervated fibres (-60 mV, Albuquerque & Thesleff, 1968; Albuquerque & McIsaac, 1970). Input resistances were significantly higher in the denervated fibres (mean $\pm$ S.D. = $2{\cdot}3 \pm 1{\cdot}1$ MΩ) than in the control fibres ($1{\cdot}0 \pm 0{\cdot}4$ MΩ). Using the short cable model (eqn. (1)), average R_m was calculated to be 1208 Ω cm^2 in the denervated fibres. This increase in membrane resistance following denervation is characteristic of frog (Nicholls, 1956) and mammalian (Albuquerque & Thesleff, 1968; Albuquerque & McIsaac, 1970) skeletal muscle.

ACh sensitivity measured at denervated end-plates ranged from several hundred to several thousand mV/nC, as at control end-plates. Extrajunctional sensitivity was unchanged one day after denervation. At 2 days, about one third of the fibres showed elevated extrajunctional sensitivity (greater than 1 mV/nC at distances greater than 200 μm from the end-plate). At longer periods after denervation, progressively higher values of extrajunctional sensitivity were observed in all fibres, and by seven days post-denervation, values exceeding 50 mV/nC could be obtained anywhere along the fibres. Beyond the region of normal extrajunctional sensitivity (150–200 μm from the end-plate), the sensitivity



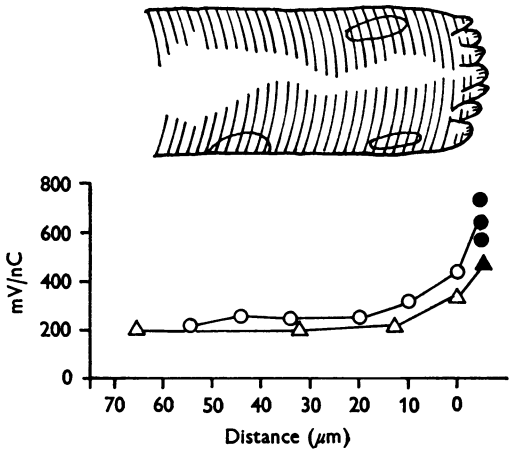
Text-fig. 7. ACh sensitivity (ordinate) at the end of a muscle fibre obtained from a muscle denervated 7 days earlier. Beyond the end-plate region, the entire fibre was uniformly sensitive to ACh.

was quite uniform. Occasionally, a gentle gradient of increasing sensitivity towards the end-plate was observed, but this was uncommon. Eighteen fibres, denervated 4–13 days previously, were mapped over 100–300 μm lengths at 5–15 μm intervals. The total length examined was 2·9 mm. No patches of membrane with significantly higher or lower sensitivities than the surround were encountered in these experiments.

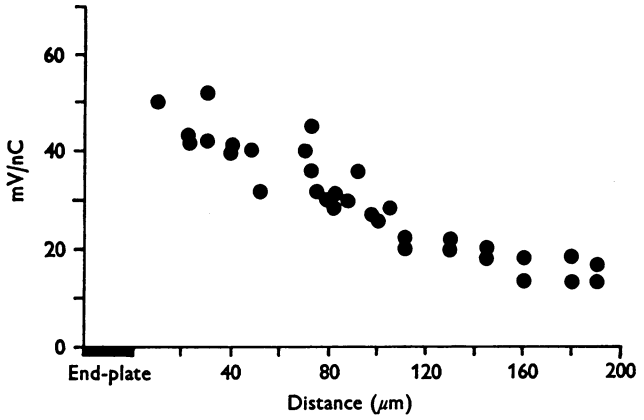
At the ends of the fibres, higher sensitivities were recorded when the ACh electrode tip was nestled amongst the folds that occur there (see Pl. 1*E*). These higher sensitivities could be due simply to the increased area of membrane exposed to ACh within the folds. An example is shown in Text-fig. 8.

The perijunctional region, normally sensitive to ACh and extending about 150 μm on either side of the end-plate, also became more sensitive to ACh after denervation. Within this region, it was possible with careful mapping to demonstrate a gradient of sensitivity, increasing towards the end-plate (Text-fig. 9). The change in sensitivity over this distance was similar to that in control fibres (typically 50–100 mV/nC over the 150 μm

distance). As in areas remote from the end-plate, no localized patches of elevated or reduced sensitivity were observed in the perijunctional region. The foregoing observations on denervated fibres can be explained by assuming that the increase in extrajunctional sensitivity in most fibres



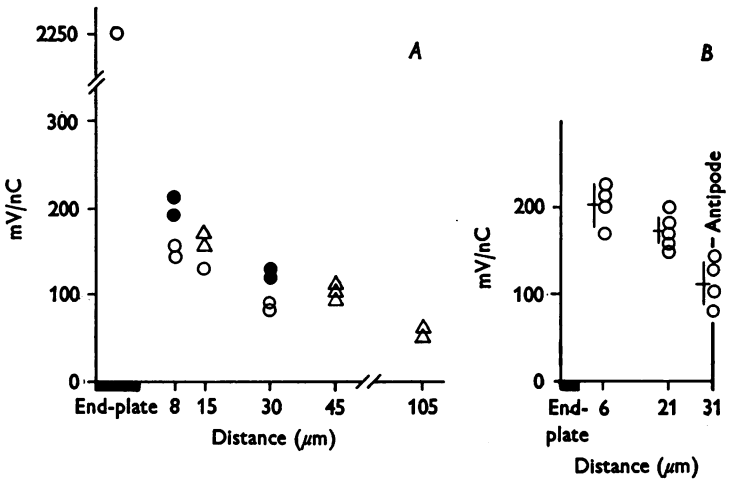
Text-fig. 8. The ACh sensitivity (ordinate) at the end of a muscle fibre denervated 7 days earlier. Filled symbols indicate sensitivities measured with ACh pipette tip nestled amongst folds at the end of fibre; other points (open symbols) were obtained along the edge of the fibre. Circles: 40 pC ACh pulses; triangles: 15 pC ACh pulses.



Text-fig. 9. ACh sensitivity (ordinate) mapped in detail on a fibre denervated 4 days earlier. At a distance of 150–200 μm from the end-plate sensitivity is 15–20 mV/nC, which is 5–10 times higher than that of control fibres. The smoothly increasing sensitivity as the end-plate is approached is still apparent.

occurs uniformly over the entire surface, and that the perijunctional receptors, present normally, persist.

The elevated levels of extrajunctional sensitivity in denervated fibres made circumferential mapping experiments more feasible. Smaller ACh iontophoretic pulses could be used to map the more sensitive membrane, and the extrajunctional ACh potentials had faster rise times (10–15 msec) in the denervated fibres. Both of these changes following denervation allowed a closer approach to the end-plate without risking contamination of the extrajunctional response by diffusion of ACh to end-plate receptors.



Text-fig. 10. ACh sensitivity (ordinate) on a single fibre from a muscle denervated 7 days earlier. *A*, fibre mapped longitudinally; *B*, fibre mapped circumferentially from end-plate. Each point represents the mean of three responses obtained at one position. Different symbols signify different iontophoretic pulse strength: open circles, 15 pC; filled circles, 26 pC; triangles, 50 pC. The lines next to the points in *B* show means and S.E. of the measurements.

Finally, perijunctional sensitivity, measured longitudinally, declined away from the end-plate in denervated fibres. All of these observations suggested that it might be possible to measure a circumferential gradient if one existed and was similar to the longitudinal gradient.

Fig. 10 illustrates results from an experiment in which extrajunctional sensitivity was mapped longitudinally (Fig. 10*A*) and circumferentially (Fig. 10*B*). Each point represents the mean of three responses (which differed from each other by less than 15 %) obtained at one position, after which the ACh electrode was moved to a new location. A circumferential gradient, similar to the longitudinal one, is clearly apparent. Similar results were obtained from two other fibres.

DISCUSSION

Collagenase digests connective tissue and tendinous insertions of skeletal muscle, and detaches end-plate cholinesterase, without affecting membrane electrical properties or ACh sensitivity (Hall & Kelly, 1971; Betz & Sakmann, 1973). The fibres are then held together loosely by interwoven nerves and vessels, and complete dissociation requires mechanical agitation, which can easily damage and break long muscle fibres. The short (*ca.* 1 mm) fibres of the flexor digitorum brevis muscle of the rat were able to withstand this treatment, and a few thousand fibres could easily be isolated in good condition.

The major difference between these fibres and undissociated mammalian muscle was the lower resting potential of the dissociated fibres (about -60 mV; undissociated fibres have resting potentials of about -75 mV). The reason for this difference is not known, but a possible explanation is that during the 2–3 hr enzyme incubation, the fibres become hypoxic, with consequent loss of activity of an electrogenic component of the Na pump. Consistent with this explanation is the observation that rat skeletal muscle fibres are rather abruptly depolarized to about -60 mV after exposure to ouabain (Bray, Hawken, Hubbard, Pockett & Wilson, 1976). Furthermore, there is evidence that a similar block occurs following denervation: denervated fibres have resting potentials of about -60 mV and they are rather insensitive to short-term exposure to ouabain (Locke & Solomon, 1967; Bray *et al.* 1976). On this basis one would expect the dissociation procedure to have little effect on denervated fibre resting potentials, which was in fact the case. Whatever the cause of the low resting potentials in control, dissociated fibres, the normal value of specific membrane resistance which was observed (500 Ω cm^2) suggests at least that a non-specific break-down in membrane permeability does not occur during the dissociation procedure.

The extrajunctional sensitivity in the control fibres is very similar to that originally described by Miledi (1960) in rat diaphragm, decreasing smoothly and progressively from the end-plate to undetectable levels over a distance of a few hundred microns. The end-plate sensitivity, and the abrupt drop in sensitivity within a distance of several microns beyond the end-plate border are similar to observations on snake and frog muscle fibres (Peper & McMahan, 1972; Dreyer & Peper, 1974*b*; Kuffler & Yoshikami, 1975).

The results of a few experiments suggested that there exists a circumferential as well as a longitudinal gradient of ACh sensitivity away from the end-plate. If these perijunctional receptors behave like the extrajunctional receptors which appear after denervation, then their distribu-

tion should be controlled by the amount of muscle activity (Lomo & Rosenthal, 1972). A circumferential gradient of activity in the end-plate region of a normal muscle cannot be ruled out, but seems rather unlikely. This raises the possibility that these ACh receptors may be subject to controls different from those regulating other extrajunctional receptors.

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EXPLANATION OF PLATES

PLATE 1

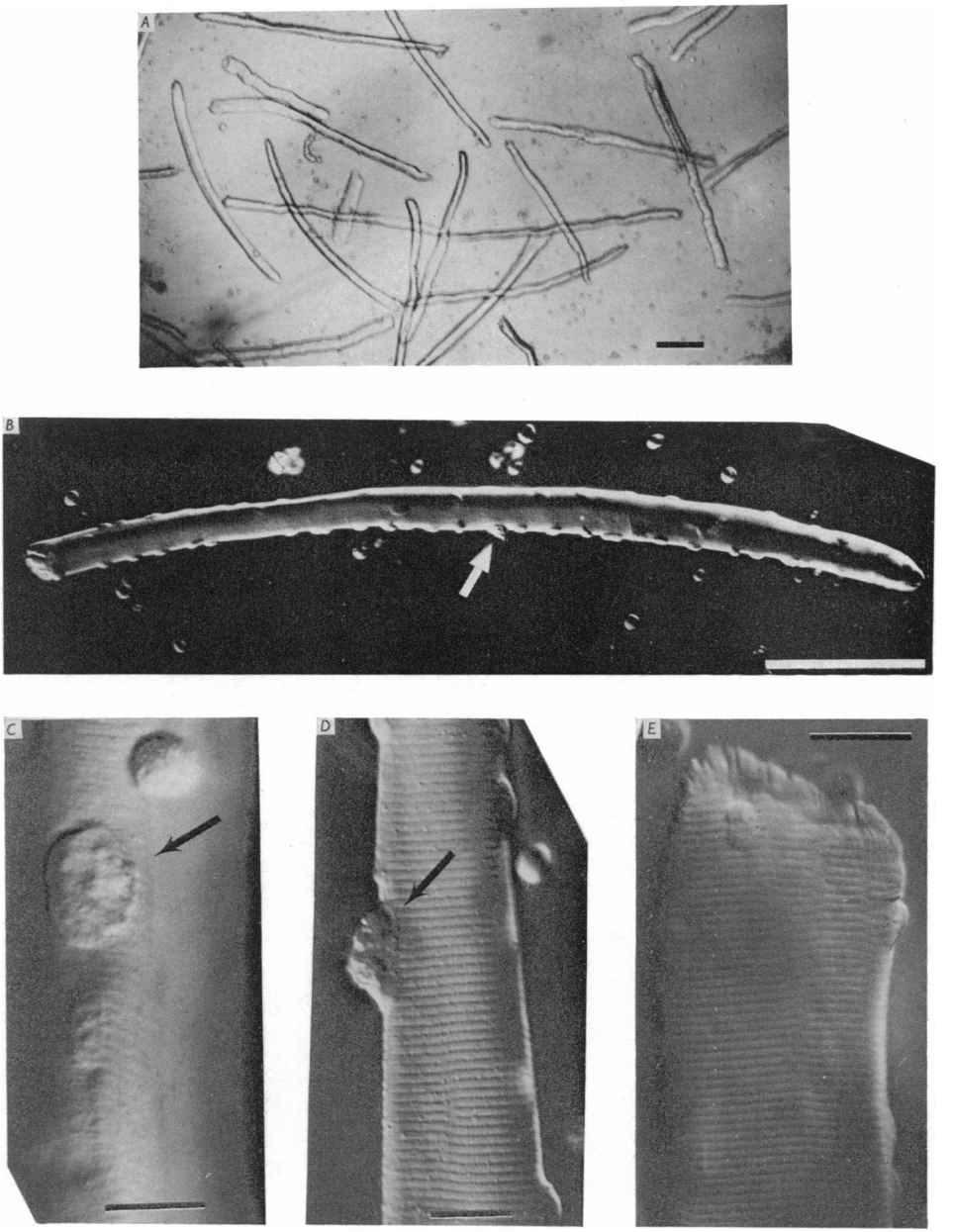
Photomicrographs of isolated flexor digitorum brevis muscle fibres. *A*, low magnification view of preparation after trituration. Calibration bar = 200 μm. *B*, higher power view of single intact muscle fibre. Nuclei bulge from its surface and the end-plate (arrow) is covered with cellular debris. Calibration bar = 200 μm. *C* and *D*, high magnification views of end-plates (arrows), one of which (*C*) appeared to be bare, and the other (*D*) covered with nerve terminal and Schwann cell debris. Calibration bars = 20 μm. *E*, the end of a muscle fibre, at which point the membrane is thrown into folds. Calibration bar = 20 μm.

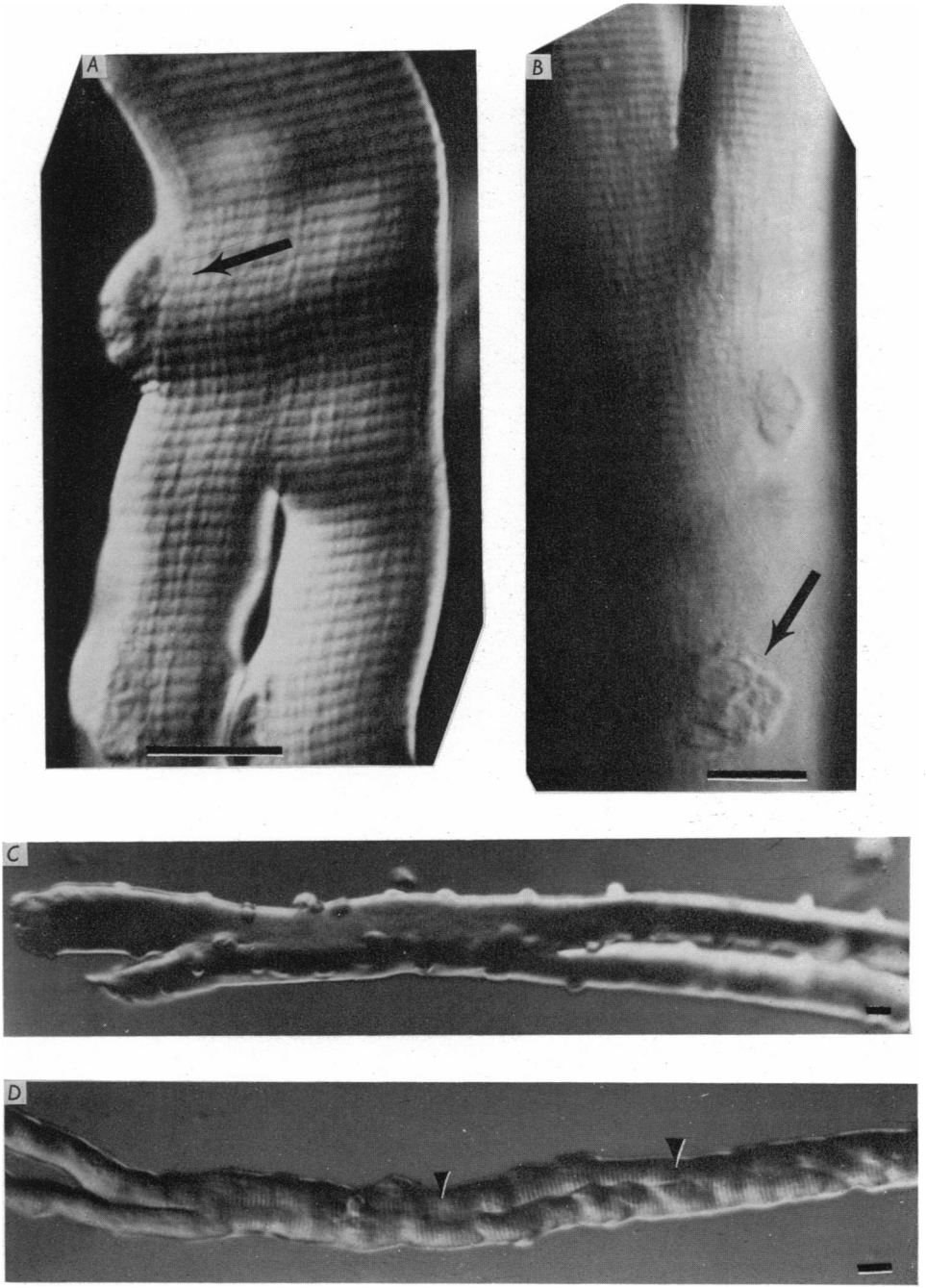
PLATE 2

Three examples of branched muscle fibres. Calibration bar = 200 μm.

PLATE 3

Branched muscle fibres. In *A* and *B*, end-plates (arrows) are located near branch points. Calibration bars = 20 μm. *C*, a doubly branched muscle fibre. Calibration bar = 20 μm. *D*, a branched muscle fibre with an additional cleft extending about 100 μm (between arrows).


A
B
C
D
E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